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Neuroprotective Effect of Environmental Enrichment on the Ultrastructure of the Optic Nerve in a Model of Type 2 Diabetes and Aging

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Abstract

Introduction: *In type 2 diabetes mellitus (T2DM), there are pathophysiological mechanisms that affect the central nervous system (CNS). Diet and sedentary lifestyle are significant risk factors for the development and progression of type 2 diabetes mellitus (T2DM) and its complications, which are exacerbated by aging and alcohol consumption. Histological modifications due to diabetes mellitus have been found in central nervous system (CNS) structures in experimental models. The optic nerve (ON) provides a model for understanding DM-related CNS damage. Environmental enrichment in experimental animals, allowing them to engage in greater physical activity and neurocognitive stimulation, could aid in the treatment and recovery of various CNS-related pathologies.*

Objective: *To study, using transmission electron microscopy, the ultrastructure of the optic nerve in diabetic experimental animals with a high-fat diet and moderate alcohol consumption, and compare it with that of animals exposed to an enriched environment.*

Materials and Methods: *Thirty-six male Wistar rats, 12 months old at the start of the trial and 24 months old at the end of the study, were divided into six groups. Their metabolism was altered by a diet containing 30% saturated fats (HFD) and/or moderate alcohol consumption (0.42 g/kg body weight/day). The enriched environment (EE) group was housed in a larger cage (250 dm^3) with running wheels and ramps, while the other animals were housed in standard animal facility cages (20 dm^3). The trial lasted 16 months. Metabolic markers were measured, and at the end of the experiment, the animals were sacrificed. The optic nerves were extracted, fixed, and processed for electron microscopy. The data obtained were analyzed using ANOVA with a $p \leq 0.05$.*

Results: *The groups fed the high-fat diet (HFD) and/or alcohol became diabetic within 7 months of the trial. Dyslipidemia and increased body weight were also observed. Animals fed the enriched environment (EA)*

showed improved glycemic levels (118 ± 5 mg/dL) and normal weight at the end of the trial. Differences in neurocognitive tests were observed among animals under different experimental conditions. Qualitative differences in the optic nerves, such as signs of atrophy and altered mitochondrial and cristae shape, were observed in those from diabetic animals fed the HFD and/or alcohol diet compared to the control groups fed the Chow diet or the Chow diet plus enriched environment. The HFD, alcohol, HFD+Alc, and HFD+Alc+EA groups showed thickening of the myelin sheaths (between 39% and 233%) compared to the control groups (Chow and Chow+EA). In all animals on the high-fat diet (HFD), more electron-dense intracytoplasmic deposits were found. In HFD+Alc and HFD+Alc+AE, the myelin sheath showed greater separation from the axon (75% and 50% more, respectively) than in group C. In the Alc group, mitochondria were observed to be larger (119 vs. 107 nm) than in the control group.

***Conclusions:** The high-fat diet and sedentary lifestyle led to the development of type 2 diabetes mellitus (DM) and neurological damage in the animals. Exposure to an enriched environment partially improved the metabolic and structural alterations of the optic nerve.*

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Keywords: Type 2 Diabetes Mellitus, Central Nervous System, Optic Nerve, High-Fat Diet, Myelin

Introduction

Type 2 diabetes mellitus (T2DM) is associated with neurobiological and cognitive deficits; its neurocognitive impact is associated with an acceleration 26% ± 14% faster than in normal aging; the duration of the disease is associated with greater neurodegeneration due to neurometabolic damage [1,2]. This has been corroborated by various studies where cognitive disabilities result in loss of functional independence [3-6]. In a previous work, we mentioned the clinical and pathophysiological characteristics of this complication, highlighting how it leads to the loss of functional independence and that, although it is a complex process involving both metabolic and vascular damage (macro and micro), it is fundamentally a metabolic process due to glycolipotoxicity, which is why it is called diabetic encephalopathy [7]. In a murine model, we described the findings of hippocampal damage and its clinical correlation [8]. A frequent, serious, and complex complication of type 2 diabetes mellitus (T2DM) is diabetic ophthalmopathy, which encompasses ocular pathologies such as cataracts, glaucoma, diabetic retinopathy, and optic neuropathy [9-11]. It is recognized as a neurovascular disease [12,13]. Cellular and molecular studies in the retinas and optic nerves of hyperglycemic and dyslipidemic experimental animals suggest that neurons and glia are vulnerable to glycolipotoxicity and are damaged after the onset of the disease, before any signs of vascular damage (14,15). Diabetic optic neuropathy is a clinical condition involving the optic pathway; in its early stages, it can be asymptomatic and can only be detected through electrophysiological measurements [16]. The relationship between glycolipotoxicity and optic nerve (ON) damage has shown morphological and morphometric lesions [17-18]. By studying the optic nerve (ON), we can observe diverse cell populations: astrocytes, oligodendrocytes, axons (both myelinated and unmyelinated), microglia, and endothelial cells. Astrocytes provide structural and metabolic support, maintain the extracellular environment, optimize neuronal signaling, and release neurotransmitters. In the ON, they provide biochemical support and maintain extracellular ionic balance for the axons of retinal ganglion cells (RGCs), which transmit retinal signals to the visual structures of the brain. Due to astrocyte-axon interactions, they participate in nerve transmission, neuronal homeostasis, and pathology [19].

Oligodendrocytes are found in regular interfascicular rows of five or more cells along the longitudinal axis of the nerve, interspersed with solitary astrocytes regularly spaced between the rows of oligodendrocytes, which extend their primary processes orthogonally and radially. These cells have a high metabolic rate since they must synthesize myelin composed of fatty acids, cholesterol, proteins, and carbohydrates. For this reason, they possess a developed metabolism with a highly developed system of endomembranes and peroxisomes, and thus be able to separate the bundles of axons into fascicles and provide the nerve with its structure by forming the myelin sheaths that facilitate the rapid conduction of the axons. [20,21,22]. In humans, the association between type 2 diabetes mellitus (T2DM) and central nervous system (CNS) involvement is observed, where a mutated gene, WFS1, encodes the multitransmembrane protein, resident in the endoplasmic reticulum (ER), called wolframin I. Its main function is to participate in ER-mitochondrial communication, specifically in the unfolded protein response (UPR). When this response is overwhelmed by various mechanisms, such as nutritional overload (whether lipids or carbohydrates), it triggers ER stress. Damage to this protein also triggers stress, leading to T2DM, deafness, cognitive impairment, and premature death, constituting Wolfram syndrome with severe oligodendrocyte involvement [23,24]. The pathogenesis of Wolfram syndrome involves abnormalities in the endoplasmic reticulum (ER) and mitochondrial dynamics. Neuroimaging studies have shown an alteration in early brain development, characterized by abnormal myelination of the white matter. Figure 1 shows how stress correlates with these conditions. Endoplasmic reticulum, mitochondrial, and cellular damage in Wolfram syndrome-related T2DM [25].

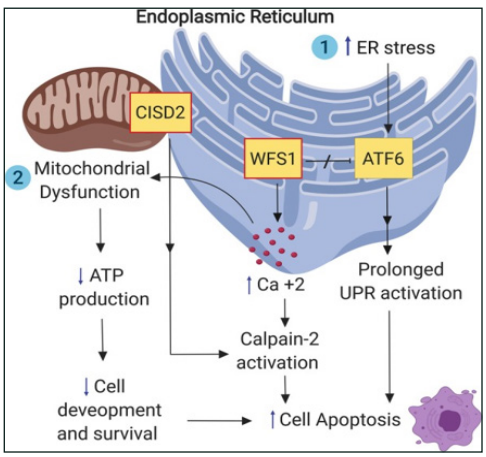


Figure 1: Schematic of the molecular changes in the endoplasmic reticulum and mitochondria in Wolfram syndrome (red box indicates deficiency of this protein). ER: endoplasmic reticulum; ATF6: activator transcription factor 6; UPR: unfolded protein response; WFS1: wolfram protein; CISD2: CISD2 protein product, ERIS [25].

Reinforcing the knowledge of the relationship of lipotoxicity, T2DM, mitochondrial damage, oligodendrocyte damage, CNS injury, Langley MR et al. observed in a murine model of T2DM based on a high-fat diet significant alterations in the CNS connectome characterized by direct impairment of mitochondrial function of oligodendrocytes, which compromises their survival, differentiation, and function [26]. In people with Alzheimer's disease (AD) without glaucoma, greater cupping of the optic nerve (ON) was observed, compared to cognitively normal people of the same age, showing that pathological processes converge in myelin damage and the coexistence of CNS and ON damage [26]. Vajaranant TS et al. used data from two ancillary studies in the Women's Health Initiative (WHI) hormone therapy trials: the WHI-Sight Exam (WHISE), conducted between 2000 and 2002, and the WHI Memory Study (WHIMS), conducted between 1996 and 2007, which provided data on vision examination, with high-quality ON photographs; Assessment of global cognitive function, systemic covariates, and lifestyle factors as risk factors for cognitive decline were collected in a randomized controlled trial. This raised the possibility of a connection between nitric oxide (NO) and brain structure and function, observing that postmenopausal women with NO damage exhibited lower global cognitive function [27]. Understanding the relationship between nutritional intake, cellular metabolism via the endoplasmic reticulum (ER), and energy transformation into ATP by mitochondria is crucial. Cell biology helps to understand the complications of type 2 diabetes mellitus (T2DM), as mitochondria and ER are connected through multiple mitochondrial membrane-associated membranes (MAMs). Alterations in mitochondrial signaling via MAMs are associated with obesity, T2DM, and neurodegenerative disorders. Disruptions in this ATP utilization process influence insulin signaling through various pathways, including Ca2+ signaling, lipid metabolism, mitochondrial function, ER stress responses, and inflammation. Altered MAM signaling is a common feature of insulin resistance (IR) in various tissues, including the liver, muscle, and central nervous system (CNS) [28].

Furthermore, from an ultrastructural morphological perspective, mitochondria can be graded according to their morphological characteristics: Grade I corresponds to normal mitochondria whose cristae complete the entire profile. Grade II shows a decreasing density of cristae, but >50% of the mitochondria is still filled with cristae. Grade III mitochondria also contain inclusion bodies. Grade IV mitochondria are filled with <50% cristae, and Grade V mitochondria are completely empty but still have a double membrane. Additionally, axons occasionally contained multilamellar structures and mitochondria containing vacuoles. In young nerves, 76% of mitochondria were Grade I or II, but higher grades became increasingly numerous in older and glaucomatous nerves. At the same time, astrocytic mitochondria appeared morphologically normal even in very old and glaucomatous nerves. Understanding mitochondrial development is fundamental in cells with a high metabolic rate, such as astrocytes and oligodendrocytes [29].

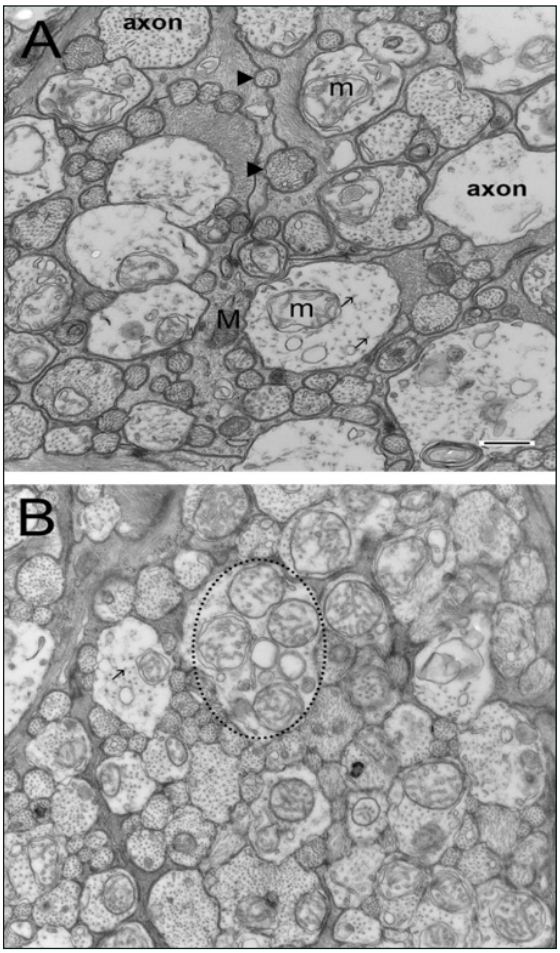


Figure 2: Enlarged, ridged axons and axonal mitochondria with loss of their cristae in the optic nerves. Shows the close structural relationship between mitochondria and myelin.
(A) Arrows point to microtubules. Solid triangles indicate normal axons. m: axonal mitochondria; M: astrocytic mitochondria.
(B) The dotted circle shows the cluster of mitochondria and cristae-less mitochondria in the abnormal axon. Scale bar: 0.5 μm. [28]

Aging is a stage of life, a biological phenomenon that we can study using animal, social, and economic models. From a biological perspective, it involves increased oxidative stress associated with the gradual degradation of nitric oxide (NO) structure and function. El-Sayyad HI et al. used one hundred male and female Wistar albino rats aged 1, 6, 18, 24, and 30 months to investigate NO using light microscopy (LM) and transmission electron microscopy (TEM), assessments of antioxidant enzymes (catalase, superoxide dismutase, and glutathione S-transferase), caspase 3 and 7, malondialdehyde, DNA flow cytometry, annexin V and CD8, immunohistochemistry of vascular endothelial growth factor (VEGF), CD31 and CD45, and single-stranded DNA fragmentation. OM and TEM observations of the older specimens (24 and 30 months) revealed axonal deterioration with NO and TEM, expressed as abundant oligodendrocytes with pyknotic nuclei, ballooned astrocytes, angiogenesis, vacuolar degeneration, and mitochondrial damage. Females were more susceptible to aging processes. The aging process was associated with: a reduction in antioxidant enzymes, increased lipid peroxidation, apoptotic markers (caspase 3 and 7, malondialdehyde, DNA flow cytometry, non-strand DNA fragmentation) and oxidative stress markers (G1, UR and LR of annexin V and CD8), a greater positive immunological reaction with VEGR, CD31, and CD45, and a decrease in antioxidant enzymes (catalase, superoxide dismutase, and glutathione S-transferase). These were related to mitochondrial damage in axons, oligodendrocytes, and astrocytes. [30]. In older adults with long-standing diabetes, phenomena associated with microangiopathy and loss of trophic support occur, since nitric oxide (NO) is highly dependent on blood flow in the peripapillary capillaries. Chronic ischemia associated with type 2 diabetes mellitus (T2DM) causes thickening of the capillary basement membrane, reducing the supply of oxygen and nutrients to the axons. Simultaneously, metabolic damage causes endoplasmic reticulum stress in oligodendrocytes, activating the GRP78/ROS pathway. This leads to myelin splitting, which can be

observed with TEM. Myelin splitting is caused by glycotoxicity, which produces non-enzymatic glycation of myelin adhesion proteins and intramyelin edema. This edema separates the myelin sheaths of astrocytes (splitting), creating spaces where fluid accumulates, thus disrupting saltatory conduction. In older adults, this results in slower vision and reduced contrast sensitivity. This causes axonal atrophy, decreased synaptophysin, and axonal shrinkage, with the consequent impairment of axonal transport, aggravated by the damage caused by oxidative stress to microtubules, preventing essential proteins such as synaptophysin from reaching synaptic terminals [31-34]. Therefore, to investigate the relationship between glycolipotoxicity, myelin impairment, mitochondrial damage, aging, the effect of an enriched environment, and social stimulation, we developed a model of nutritional impairment of nitric oxide (NO) levels using a diet rich in saturated fats and the effect of an environment enriched with physical and social activity.

Materials and Methods

Materials and Methods: Thirty-six male Wistar rats, 12 months old at the start of the trial, were divided into six groups. To alter their metabolism, a diet containing 30% saturated fat (HFD) and/or moderate alcohol consumption (0.42 g/kg body weight/day) was used. The enriched environment (EE) group was housed in a larger cage (250 dm^3) with running wheels and ramps, while the other animals were housed in standard animal facility cages (20 dm^3). The trial lasted 16 months. Clinical and metabolic markers were measured. At the end of the experiment, the animals were sacrificed, and their optic nerves were extracted, fixed, and processed for electron microscopy. The involvement of the mitochondrial cristae and the number of deposits in the cytoplasm of the axons were classified in a severity order from 0 to 5. The data obtained were analyzed by ANOVA with a $p \leq 0.05$.

Morphometry

All myelinated and unmyelinated nerve fibers were counted in images of ten random areas of semi-thin sections obtained from each nerve (2000X magnification). Thirty nerves from hyperglycemic animals and 30 control nerves were used in this morphometric study. No blinding was used. Axon and fiber diameters in each image were measured using an image analyzer (Image Pro v6.0, Media Cybernetics®, Maryland, USA). Myelin sheath thicknesses were estimated based on these measurements.

EXPERIMENTAL DESIGN				
Group	N	Diet	Water	Habitat
Control	7	Standard Chow diet for laboratory animals (GEPSA Feeds. Grupo Pilar S.A. Córdoba, Argentina)	Water	Standard Box
				(20 cm3)
Enriched Environment (EE)	6	Standard Chow diet for laboratory animals (GEPSA Feeds. Grupo Pilar S.A. Córdoba, Argentina)	Water	Large box (250 dm3) enriched environment with wheels, platforms, ramps, physical activity, etc.
Alcohol	6	Standard Chow diet for laboratory animals (GEPSA Feeds. Grupo Pilar S.A. Córdoba, Argentina)	Water + 96% Ethanol (0.42 g/kg of body weight per day)	Standard Box
				(20 cm3)

High Fat Diet (HFD)	6	Chow diet + 250g saturated fats/kg diet (diet contains approximately 30% w/w fats, 25% saturated)	Water	Standard Box
				(20 cm3)
HFD+Alc	6	Chow diet + 250g saturated fats/kg diet (diet contains approximately 30% w/w fats, 25% saturated)	Water + 96% Ethanol (0.42 g/kg of body weight per day)	Standard Box
				(20 cm3)
HFD+Alc+AE	6	Chow diet + 250g saturated fats/kg diet (diet contains approximately 30% w/w fats, 25% saturated)	Water + 96% Ethanol (0.42 g/kg of body weight per day)	Large box (250 dm3) enriched environment with wheels, platforms, ramps, physical activity, etc

Morphometric Analysis at the Mitochondrial Level

To compare changes in mitochondria, we used a five-grade classification. Grade I: normal, full of cristae; Grade II: some loss of cristae, still >50% full; Grade III: mitochondria contain inclusion bodies; Grade IV: mitochondria have lost almost all cristae; Grade V: lack cristae, have a double membrane, multilamellar bodies, and vacuoles.

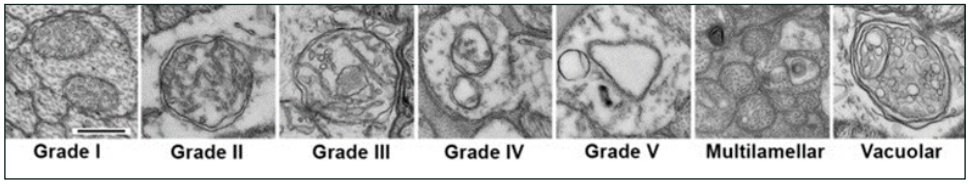


Figure 3: Schematic classification of mitochondrial damage into five grades [29].

Statistical Studies

At the end of the experiment, the animals were sacrificed, and the optic nerves were extracted, fixed, and processed for electron microscopy. The data obtained were analyzed using ANOVA with $p \leq 0.05$.

Results

The groups with a high-fat diet (HFD) and/or alcohol consumption developed diabetes within 7 months of the trial. Dyslipidemia and increased body weight were also observed. Animals with an elevated alcohol content (AEC) showed improved glycemic levels (118 ± 5 mg/dL) and normal weight at the end of the trial. Differences were observed in neurocognitive tests among the animals under different experimental conditions. Qualitative differences were observed in the optic nerves of diabetic animals fed the HFD diet and/or alcohol, such as signs of atrophy and alterations in the shape of mitochondria and their cristae, compared to those of the control groups fed the Chow diet or the Chow diet plus an enriched environment. The HFD, Alc, HFD+Alc, and HFD+Alc+AE groups showed thickening of the myelin sheaths (between 39% and 233%) compared to the control groups (C and C+AE). In all animals fed the HFD diet, more electron-dense intracytoplasmic deposits were found. In the HFD+Alc and HFD+Alc+AE groups, the myelin sheath was observed to be more separated from the axon (75% and 50% more, respectively) than in group C. In the Alc group, the mitochondria were larger (119 vs. 107 nm) than in the control group.

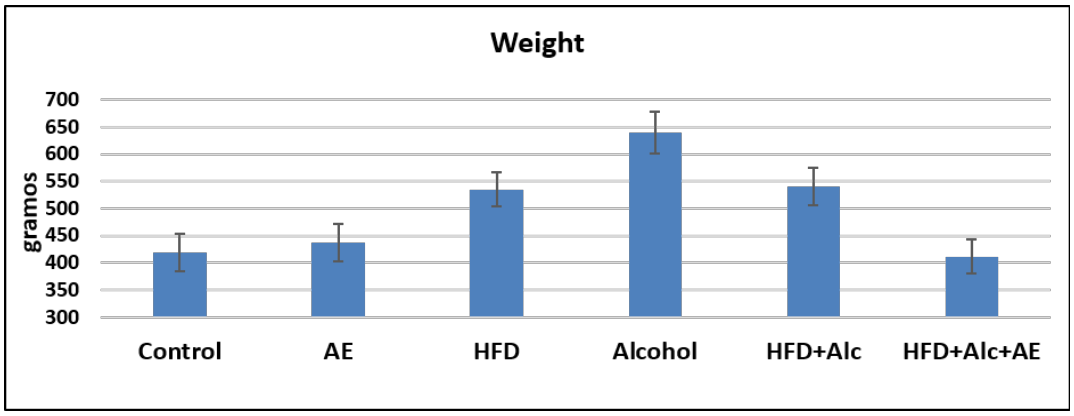


Figure 4: Anthropometric results Body weight in the different groups studied

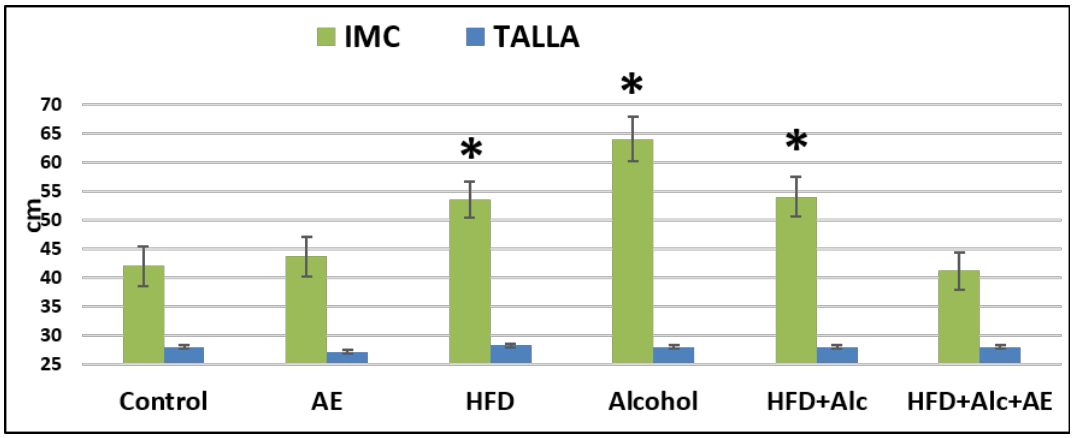


Figure 5: Anthropometric results: Body Mass Index and Height in the different groups studied

*indicates a significant difference ($p \leq 0.05$) compared to the control group

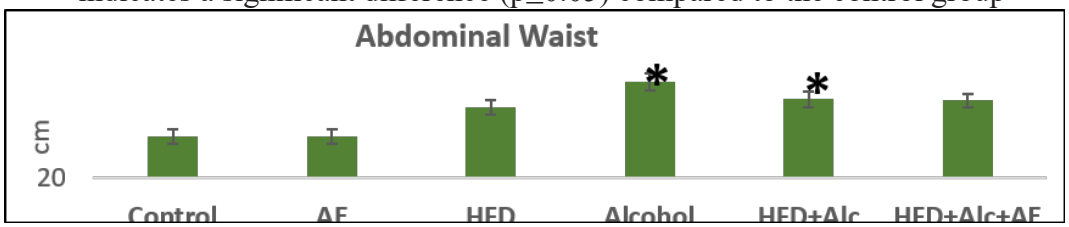


Figure 6: Anthropometric results: Abdominal Waist circumference in the different groups studied.

*Indicates a significant difference ($p \leq 0.05$) compared to the control group.

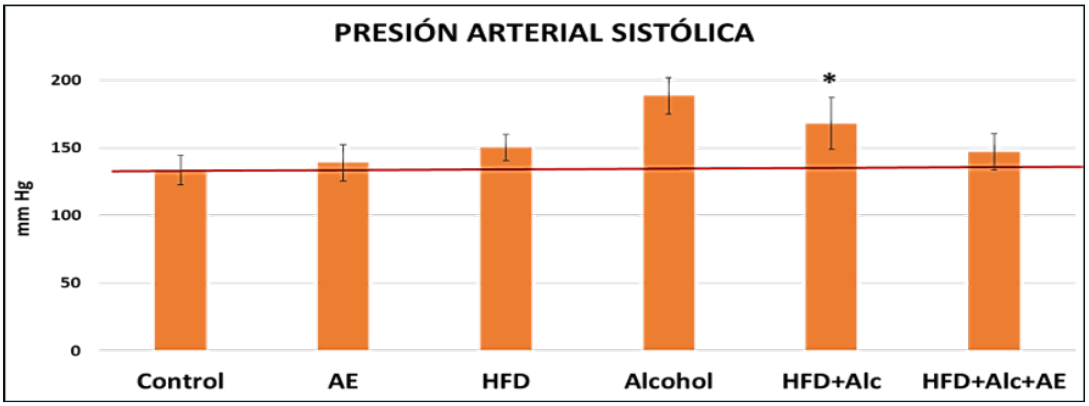


Figure 7: Results of systolic blood pressure measurement in the different groups studied

*indicates a significant difference (p≤0.05) compared to the control group

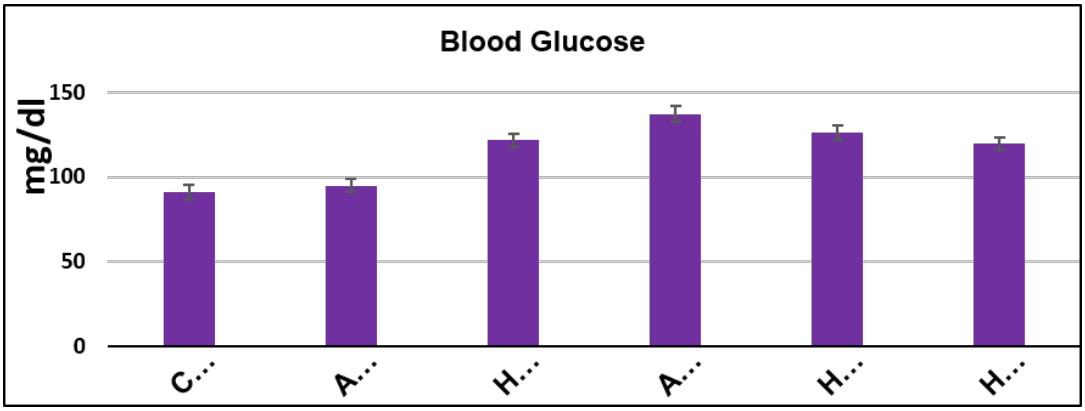


Figure 8: Blood glucose results in the different groups studied

*indicates a significant difference (p≤0.05) compared to the control group

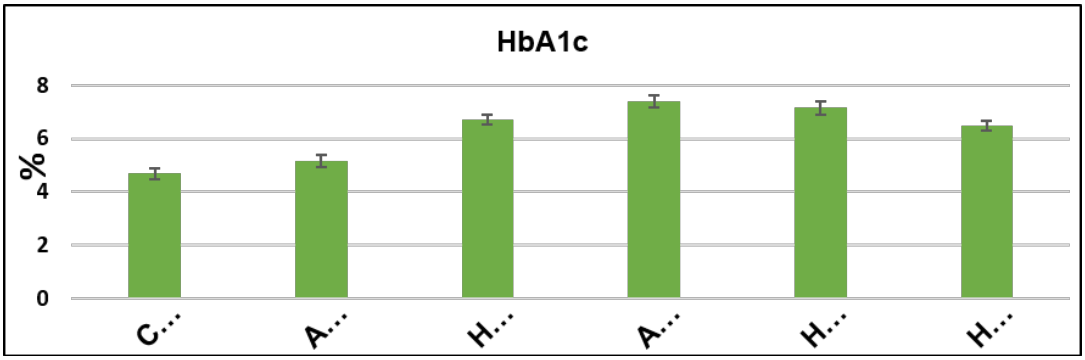


Figure 9: Glycated hemoglobin (HbA1c) results in the different groups studied

*indicates a significant difference (p≤0.05) compared to the control group

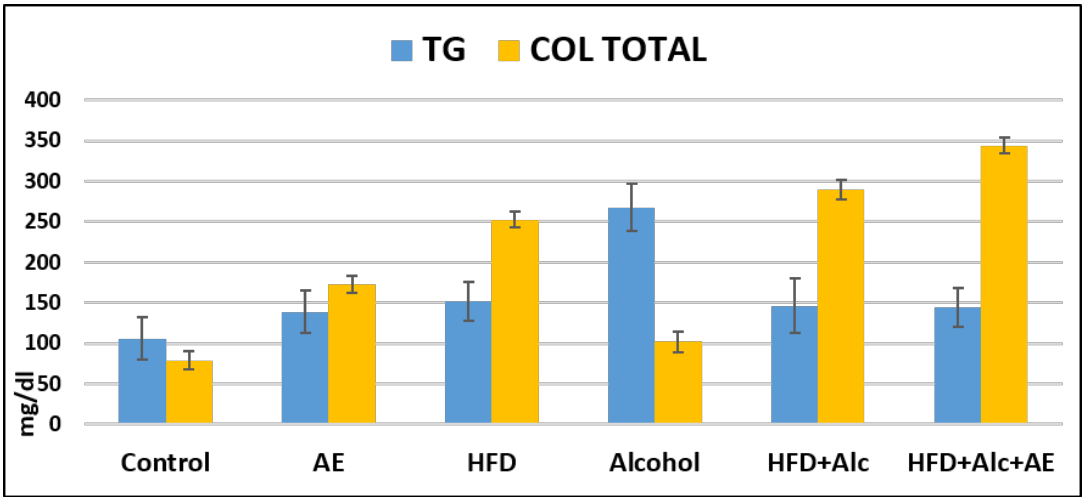


Figure 10: Laboratory results. Triglyceride and total cholesterol values in the different groups studied.

Structures and Ultrastructures of Nerve Fibers.

In the optic nerve studies, a group of young control animals (3 months, n=3) was added to differentiate the effects of aging from those produced by metabolic syndrome.

*Indicates a significant difference ($p \leq 0.05$) compared to the control group

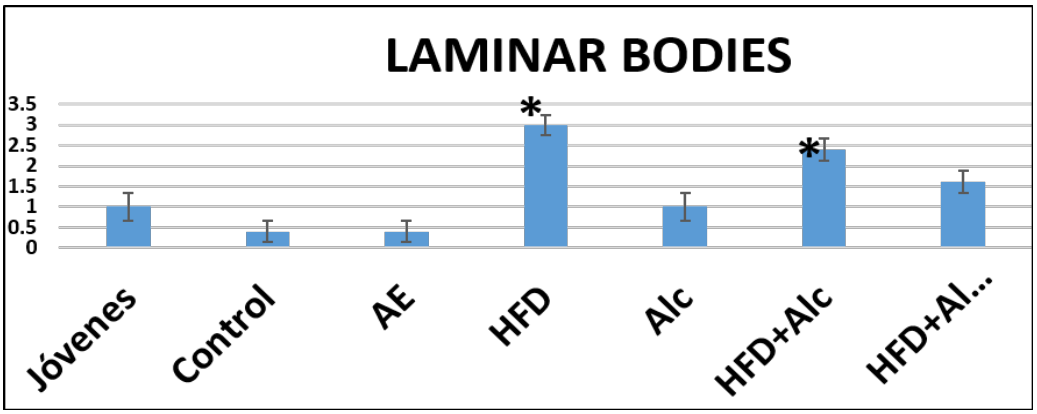


Figure 11: Observation of the number of lamellar bodies per field in transmission electron microscopy (TEM) images at 2,000x magnification

*indicates a significant difference ($p \leq 0.05$) compared to the control group

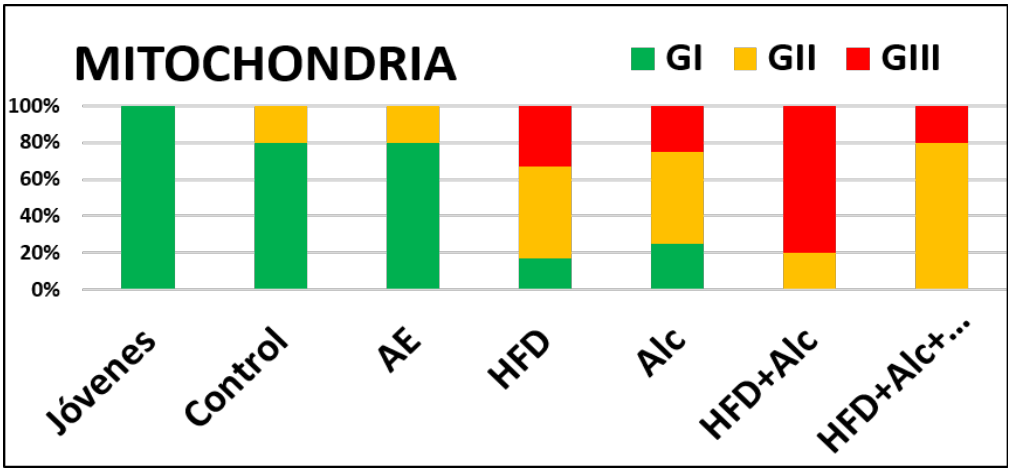
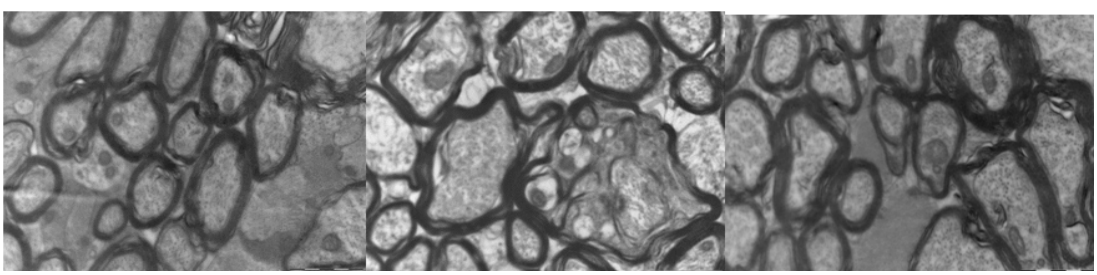


Figure 12: Observation of the degree of mitochondrial injury, according to Zhu et al. [29] per field of transmission electron microscopy (TEM) images at 2000x magnification.

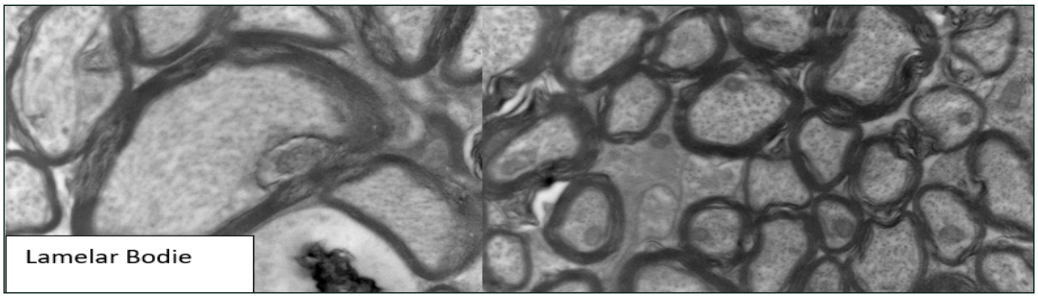
*Indicates a significant difference ($p \leq 0.05$) compared to the control group.

In the control animals, myelinated and unmyelinated fibers formed bundles shared with glial cell processes and blood vessels. The fiber axoplasms were light and contained mitochondria, microtubules, and neurofilaments. In the HFD and HFD + alcohol-treated groups, ballooned mitochondria with loss of their cristae were observed. Furthermore, a considerable number of axons were surrounded by an internal mesaxon structure. Myelin collapse was frequently observed, and lamellar membranous bodies were also frequently seen in hyperglycemic animals.

Images



Control Alcohol HFD



HFD + Alcohol HFD Enriched Environment

Figure 13: Transmission Electron Microscopy images corresponding to axons in which myelin with separation is observed

Discussion

To understand optic neuropathy due to glycolipotoxicity, we must understand the structure and function of oligodendrocytes (OLs). They coat the axons of the central nervous system (CNS) with myelin, a compact and highly organized multimembrane structure. Myelination allows the rapid transmission of action potentials and preserves axonal integrity through various mechanisms, including metabolic support of OLs and astrocytes [36]. Thus, the loss of myelin in diseases of diverse etiologies, such as multiple sclerosis, Alzheimer's disease (AD), and type 2 diabetes (T2D), causes axonal degeneration and clinical deterioration in patients by affecting the cellular metabolism of cell populations in the CNS and pancreas [37, 38]. In postnatal development, OL progenitor cells (OPCs) undergo a tightly orchestrated differentiation program that leads to mature OLs coming into contact with axons and wrapping them with myelin [39]. A population of OPCs remains resident in the adult CNS, called adult OPCs (aOPCs) [40]. OPCs are activated by demyelinating injury, migrate to the injury site, proliferate, differentiate, and mature into OLs that remyelinate axons, replacing the pre-existing ones lost with demyelination [41]. This mechanism fails in chronic processes such as type 2 diabetes mellitus (T2DM), resulting in the generation of myelin, leaving axons particularly vulnerable [42,43]. Myelin is characterized by an exceptionally high lipid content (~80% of dry weight) [44]. Fatty acids (FAs) are fundamental components of glycolipids and phospholipids, which constitute the largest proportion of lipids in the myelin membrane [45]. Essential and non-essential FAs originate from the circulation due to dietary intake, cross the blood-brain barrier, or are synthesized endogenously in cells [46].

In our study, we used a model based on a high-fat diet (HFD) [22] to develop a metabolic syndrome that led to type 2 diabetes mellitus (T2DM). This syndrome worsened when working with older animals (over one yearold), and in a group where alcohol was added, this substance exacerbated the metabolic and blood pressure conditions.

When studying the presence of lamellar bodies, also known as redundant myelin figures or myelinated bodies (Fig. 11), we observed a significant increase in the HFD group and the group that associated a high-fat diet with alcohol, and a positive effect of the enriched medium with a decrease in the frequency of this complication. This ultrastructural finding is characteristic and indicates the severity of diabetic neuropathy. These are concentric whorls or spirals of densely packed myelin membranes, constituting membranous debris. They form when the myelin sheath detaches from the axon and collapses upon itself, due to glycation of plasma membrane proteins, possibly Myelin Basic Protein (MBP). This would be closely related to endoplasmic reticulum stress in oligodendrocytes, which increases GRP78/ROS levels (which could not be measured). Incomplete autophagy is also present, as the cell fails to recycle damaged membranes. This would be associated with: demyelination, Wallarian degeneration, failure in remodeling (indicates that the animal's natural repair mechanisms are overwhelmed by metabolic stress) [47-50].

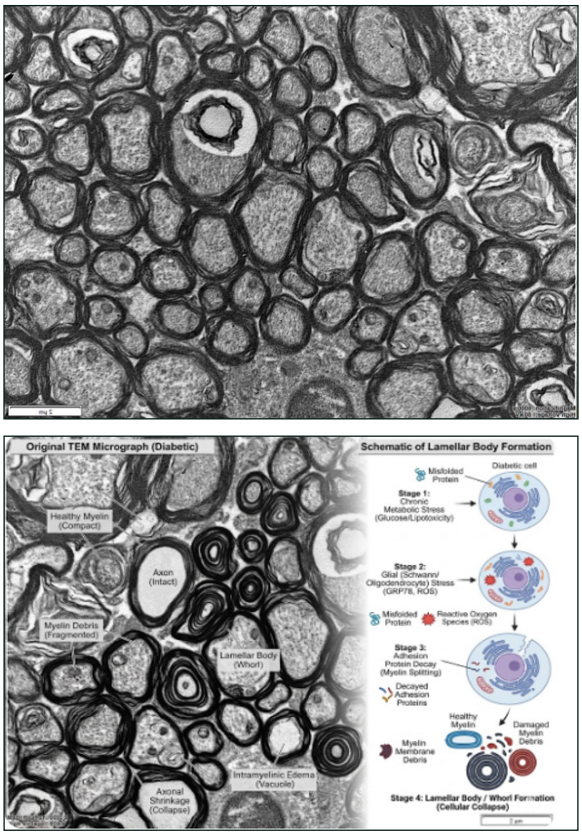


Figure 14: A TEM image of an animal from the HFD Group showing lamellar bodies. B Analysis and diagram by Gemini 5.

By analyzing the mitochondria (Fig 13), we were able to quantify their lesions. We highlight that the young group fed a chow diet had no lesions, while the "older adult" group presented an estimated 20% mitochondrial involvement. This would be related to the effect that aging produces on mitochondria. Ozgen et al. provide an elegant review of the topic, highlighting that mitochondria play a multidimensional role in the function and vitality of the neurological system. They generate neural stem cells, maintain neural cell populations by regulating homeostasis—a crucial task for cognitive health and neurological well-being—and provide energy through oxidative phosphorylation for neurotransmission and the generation of an action potential along the neuron's axon. This process is affected by T2DM [51]. In the study groups, we see how a high-fat diet (HFD) and alcohol, individually, are harmful to mitochondrial function and structure, and how their combination exacerbates the damage. The enriched medium plays an important role in mitigating this damage, as we can observe in the findings of this study. Recent work shows that the stimulation of SIRT3, a key mitochondrial deacetylase described as the mitochondria's "energy sensor," regulates cellular function by controlling the production of reactive oxygen species (ROS), metabolism, and cellular integrity. Furthermore, it has a dual role as a tumor suppressor or cancer promoter depending on the context. This would be the key site where the enriched medium would act [52-54].

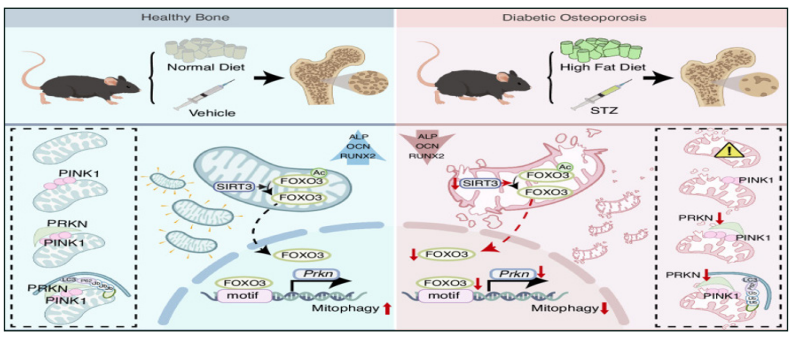


Figure 15: Stimulation of SIRT3 in a model of diabetes and osteoporosis shows that this cellular pathway, when stimulated in different ways, could explain how the enriched medium might improve mitochondrial function.

TEM of NO allowed observation of a decrease in the number of axons, separation of myelin layers and a decrease in their thickness, and deposition of lamellar bodies. The separation of the myelin sheath layers would be due to failures in the adhesion proteins, accumulation of fluid between the myelin sheets, causing edema, retraction of the axolemma, reduction of the axon diameter, indicating a loss of cytoplasmic volume, electron-dense accumulations of damaged organelles or debris within the axon which constitutes a sign of degeneration, a general process of disintegration of the axonal and myelin structure [13], vacuoles which are empty spaces or bubbles that indicate cell death or ballooning [55-57.] Schematic in Fig16,17 and 18.These lesions were observed in all groups even in the control group which although they did not receive HFD but were "older adults in this case the damage was an expression of the aging process. In the Alcohol Group, myelin fragmentation and delamination were observed as an effect of even small doses of ethanol, which was exacerbated in the group receiving a high-fat diet plus alcohol. In the Medium Enriched Group, while lesions were not prevented, they were mitigated in both the control and high-fat diets, demonstrating the importance of mobility and social interaction.

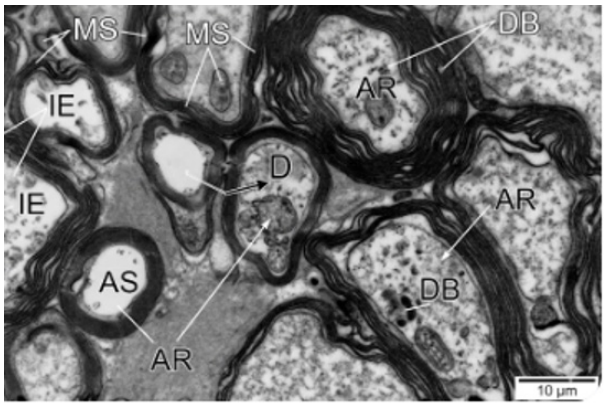
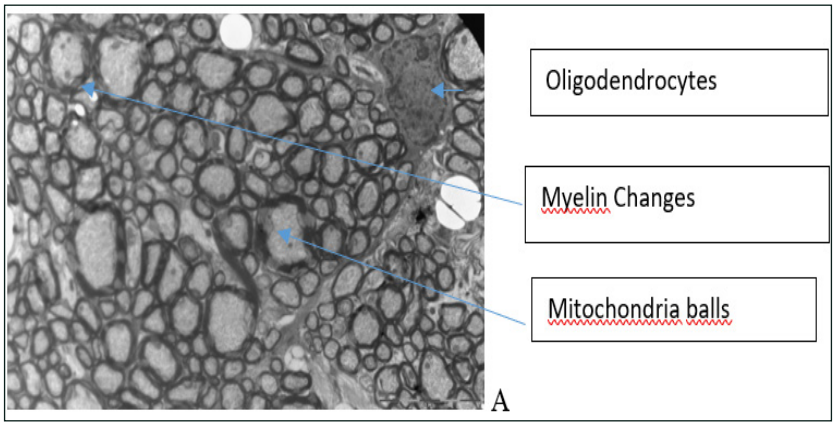


Figure:16 Effect of High Fat Diet (HFD)
MS: Myelin Splitting, IE: intramyelinic edema, AR: Axoloemma Retraction, AS: Axonal Shrinkage, DB: Dense Body
D: Degeneration



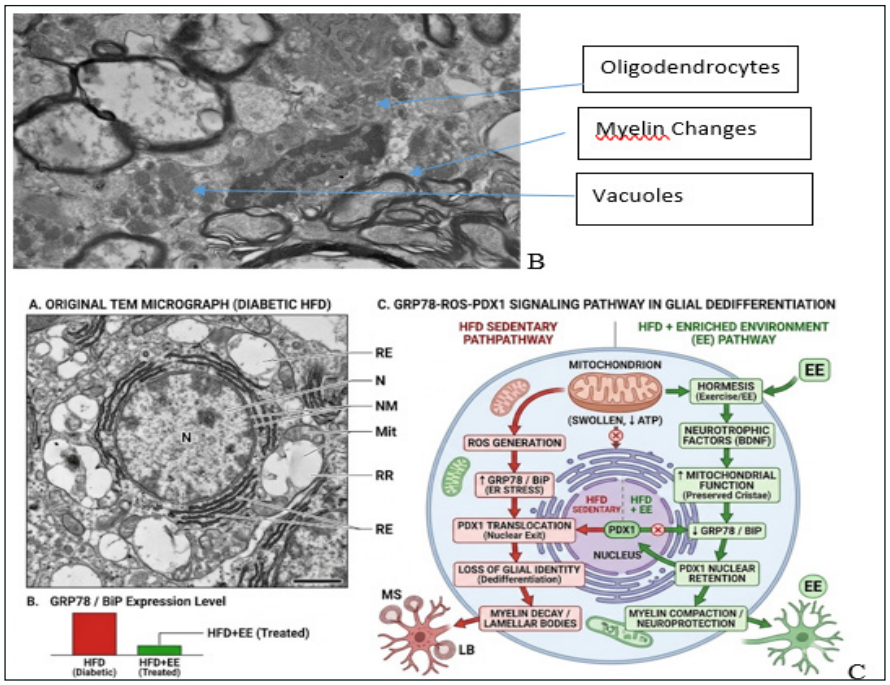


Figure 17: Scheme of NO HFD+ME group shows how an oligodendrocyte surrounds the axons and the main pathological changes.

A 2000x B 6000x C Diagram by Gemini showing structural modifications and possible pathophysiological changes

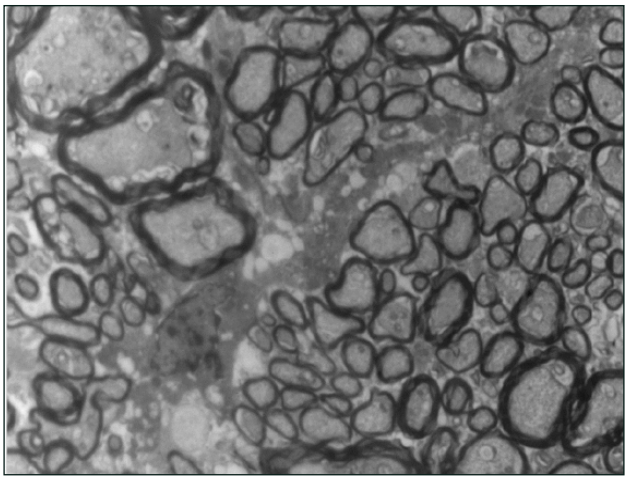


Figure 18: MET of NO HFD Group shows how an oligodendrocyte surrounds the axons, its nuclear membrane is affected, numerous vacuoles inside, mitochondrial damage in axons and oligodendrocyte, myelin delamination and lamellar bodies.

When we analyze the data obtained from this work from the perspective of the impact on older adults with metabolic syndrome and type 2 diabetes, we find that to understand its clinical manifestations, animal models can reflect equivalent findings such as weight gain in relation to saturated fat and alcohol intake (Figs. 4, 5), where the weight gain is located in the abdomen, the site of metabolically active fat. This is related to the "Western diet," closely linked to diabesity, and how lifestyle modification would help in its treatment [58]. We saw how diabesity is related to increased glycemic values, HbA1c, and triglycerides (Figs. 8, 9). 11 and the close relationship with hypertension [59]. The damage observed in the optic nerves can be understood as an effect of dietary injury and aging, elements that negatively reinforce each other, as Hayden [60] describes. Furthermore, an enriched environment, understood as greater mobility and social interaction, has a beneficial effect on

this damage, with low complications and costs in an aging society with increasingly fewer resources for healthcare.

Thus, our findings on hyperglycemic, hypertriglyceridemic, sedentary, and aged animals induced by HFD can contribute to understanding the pathogenesis of optic neuropathy in obesity, metabolic syndrome, and diabetes. Aging, due to increased mitochondrial damage and oxidative stress, constitutes a common risk factor for diabetes and neurodegenerative diseases [61-64].

Conclusions

A high-fat diet and sedentary lifestyle led to the development of diabetes mellitus and neurological damage in the animals. Exposure to an enriched environment partially improved the metabolic and structural alterations of the optic nerve.

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